Supplementary information

Structural and functional characterization of the Curli adaptor protein CsgF

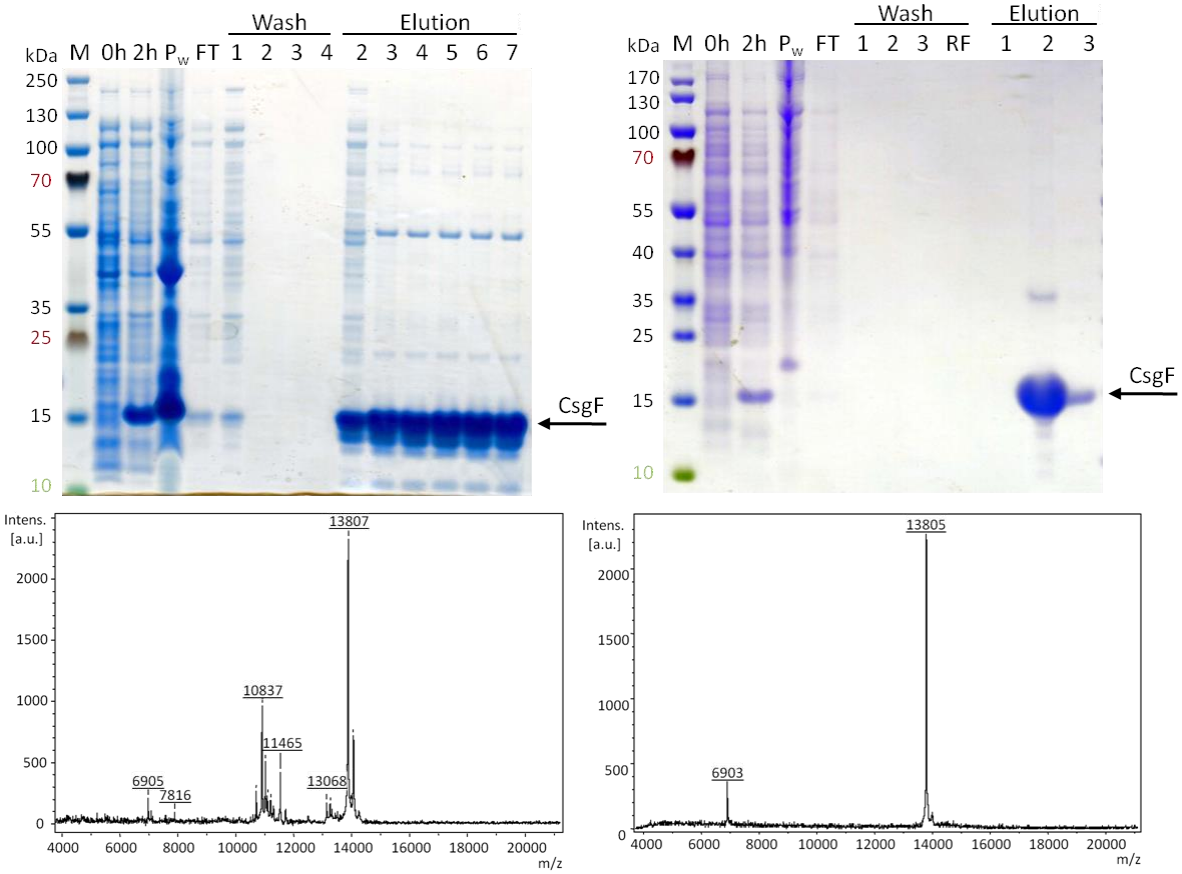


Figure S1: Native (left) and denaturing/refolding (right) purification of His tagged CsgF analyzed by SDS-PAGE. M: protein marker; Full cell samples at 0h/2h time of protein expression; Pw: washed sample of insoluble cell debris after lysis; FT: flow through fraction of Ni-affinity chromatography; Wash: fractions of washing steps; RF: fraction of refolding step; Elution: fractions of elution steps. The eluted proteins were evaluated with MALDI mass spectrometry.

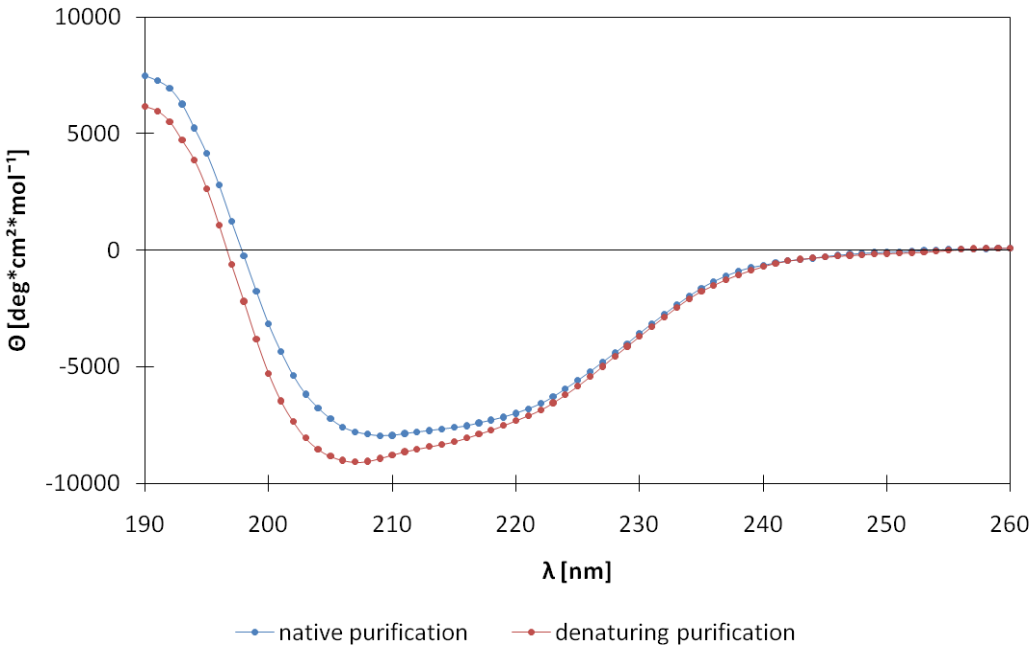
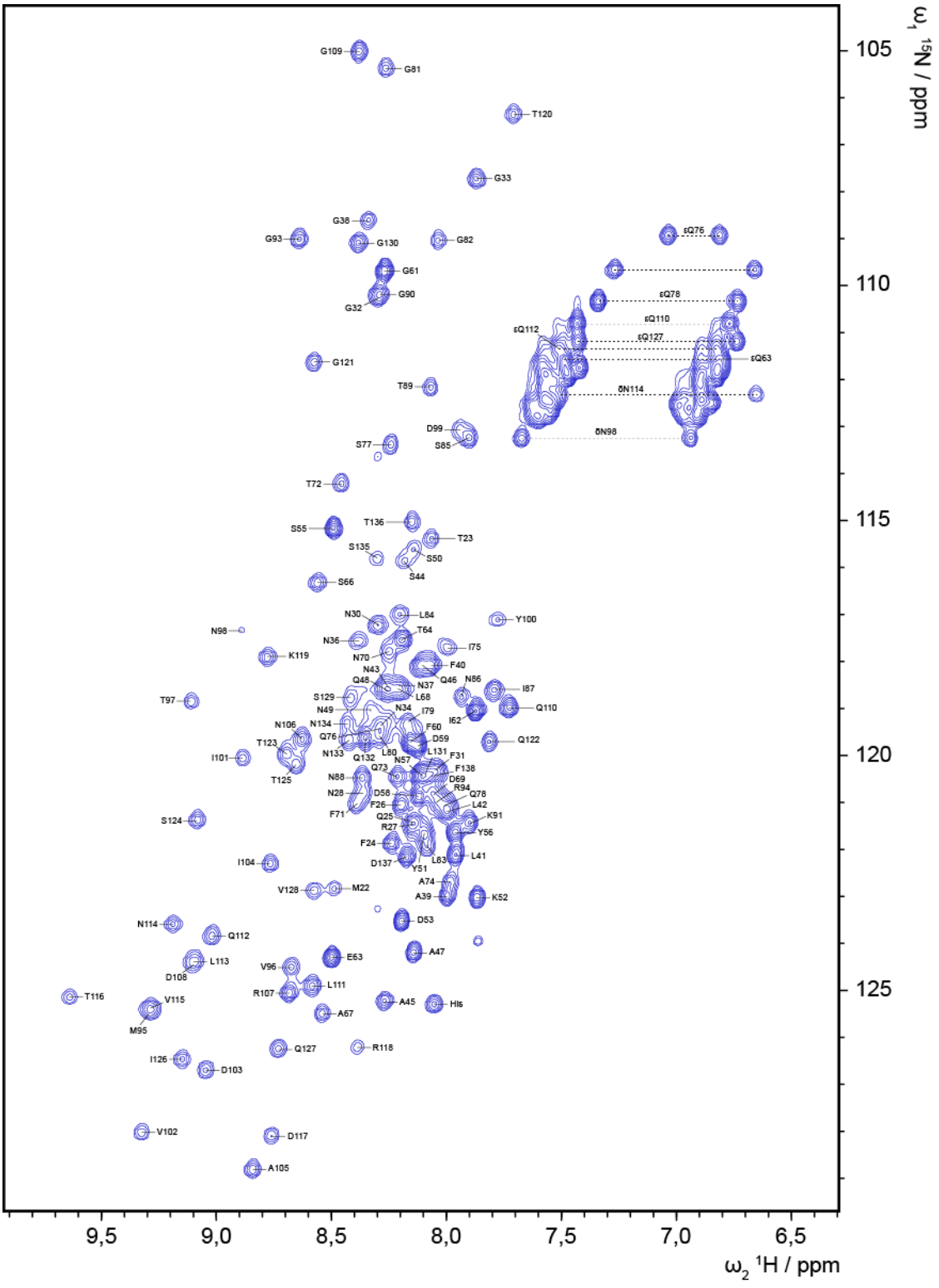


Figure S2: Far-UV spectra of CsgF purified under native and denaturing conditions. Spectra were recorded in 50 mM Phosphate Buffer at pH 7.

Table S1: Parameters of three dimensional NMR spectra, recorded at 600 MHz.

Experiment	*t1 (ms)*	*t2 (ms)*	*t3 (ms)*	*Mix (ms)*	*Scans*	*Ref.*
HNCO	17.6 (^{13}C)	17.9 (^{15}N)	121.7 (^{1}H)	-	8	(Kay et al. 1990)
HN(CA)CO	22.0 (^{13}C)	20.9 (^{15}N)	121.7 (^{1}H)	-	16	(Clubb et al. 1992)
CBCA(CO)NH	6.2 (^{13}C)	17.9 (^{15}N)	121.7 (^{1}H)	-	12	(Grzesiek and Bax 1992)
HNCACB	7.6 (^{13}C)	17.9 (^{15}N)	121.7 (^{1}H)	-	16	(Grzesiek and Bax 1992)
(H)CC(CO)NH	6.1 (^{13}C)	20.3 (^{15}N)	53.3 (^{1}H)	20	32	(Montelione et al. 1992)
^{15}N edited TOCSY-HSQC	20.1 (^{1}H)	20.3 (^{15}N)	56.8 (^{1}H)	50	16	(Marion et al. 1989a)
^{13}C edited TOCSY-HSQC	20.9 (^{1}H)	6.6 (^{13}C)	113.6 (^{1}H)	60	8	(Bax et al. 1990)
^{13}C-HSQC-NOESY-^{15}N-HSQC	6.4 (^{13}C)	20.3 (^{15}N)	60.9 (^{1}H)	80	24	(Diercks et al. 1999)
^{15}N edited NOESY-HSQC	20.1 (^{1}H)	28.7 (^{15}N)	56.8 (^{1}H)	80	8	(Marion et al. 1989b)
^{13}C edited NOESY-HSQC (Ali)	20.9 (^{1}H)	7.0 (^{13}C)	42.6 (^{1}H)	80	8	(Marion et al. 1989b)
^{13}C edited NOESY-HSQC (Aro)	21.3 (^{1}H)	6.0 (^{13}C)	85.2(^{1}H)	80	4	(Marion et al. 1989b)



18 21 31 41 51 61 71 81
MAG TMTFQFRNPN FGGNPNNGAF LLNSAQAQNS YKDPSYNDDF GIETPSALDN FTQAIQSQIL GGLLSNINTG

91 101 111 121 131 141
KPGRMVTNDY IVDIANRDGQ LQLNVTDRKT GQTSTIQVSG LQNNSTDFHH HHHH

Figure S3: ^{1}H-^{15}N HSQC Spectrum of 400 µM CsgF annotated with the resonance assignments (top), amino acid sequence of CsgF (bottom), unassigned residues are colored in red

Table S2: Statistics of NMR structure calculation and refinement.

NMR constraints	
Total NOE distance restraints	1027
Intraresidue, i – j = 0	368
Sequential, i – j = 1	386
Medium range, 1 < i – j < 5	144
Long range, i – j ≥ 5	129
Dihedral angle restraints	515
Structural Statistics	
Average violations per structure	
NOEs ≥ 0.1 Å	6 ± 2
Dihedrals > 2°	19 ± 3
RMSD (Residues 68 – 127)	
Backbone (Å)	5.77 ± 1.75
Heavy (Å)	6.28 ± 1.77
Ramachandran Analysis	
Most favored region	65.2
Allowed region	28.6
Generously allowed	2.8
Disallowed	3.4
BMRB accession number	34052
PDB ID code	5M1U

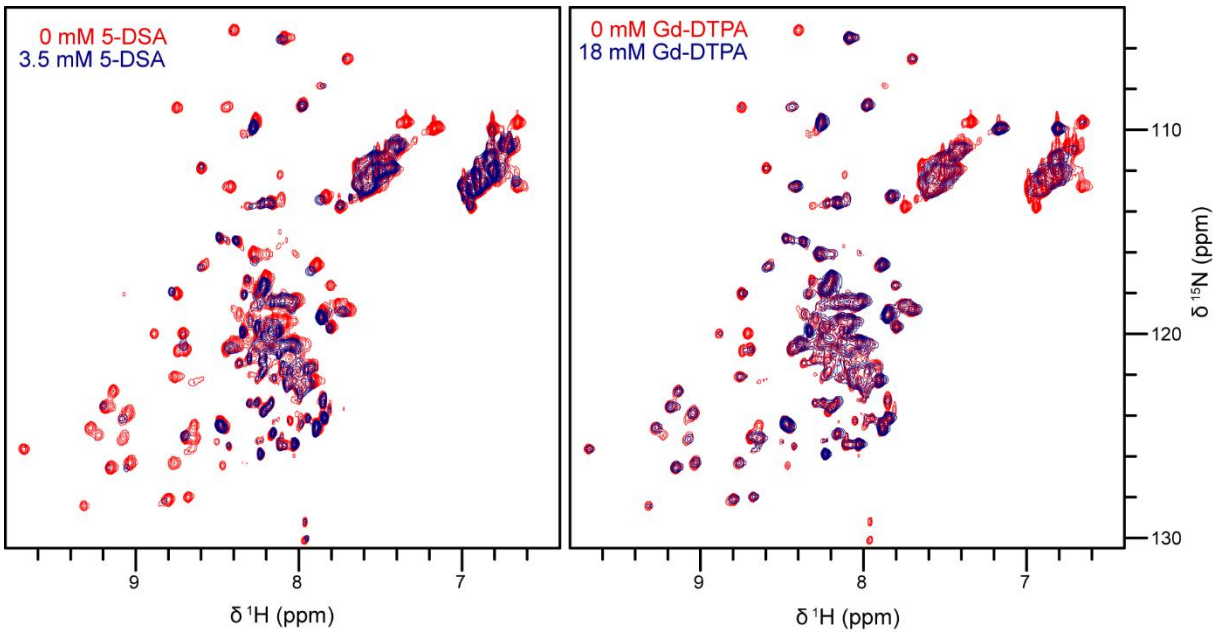


Figure S4: ^{1}H-^{15}N HSQC Spectra, Titration of paramagnetic substances.

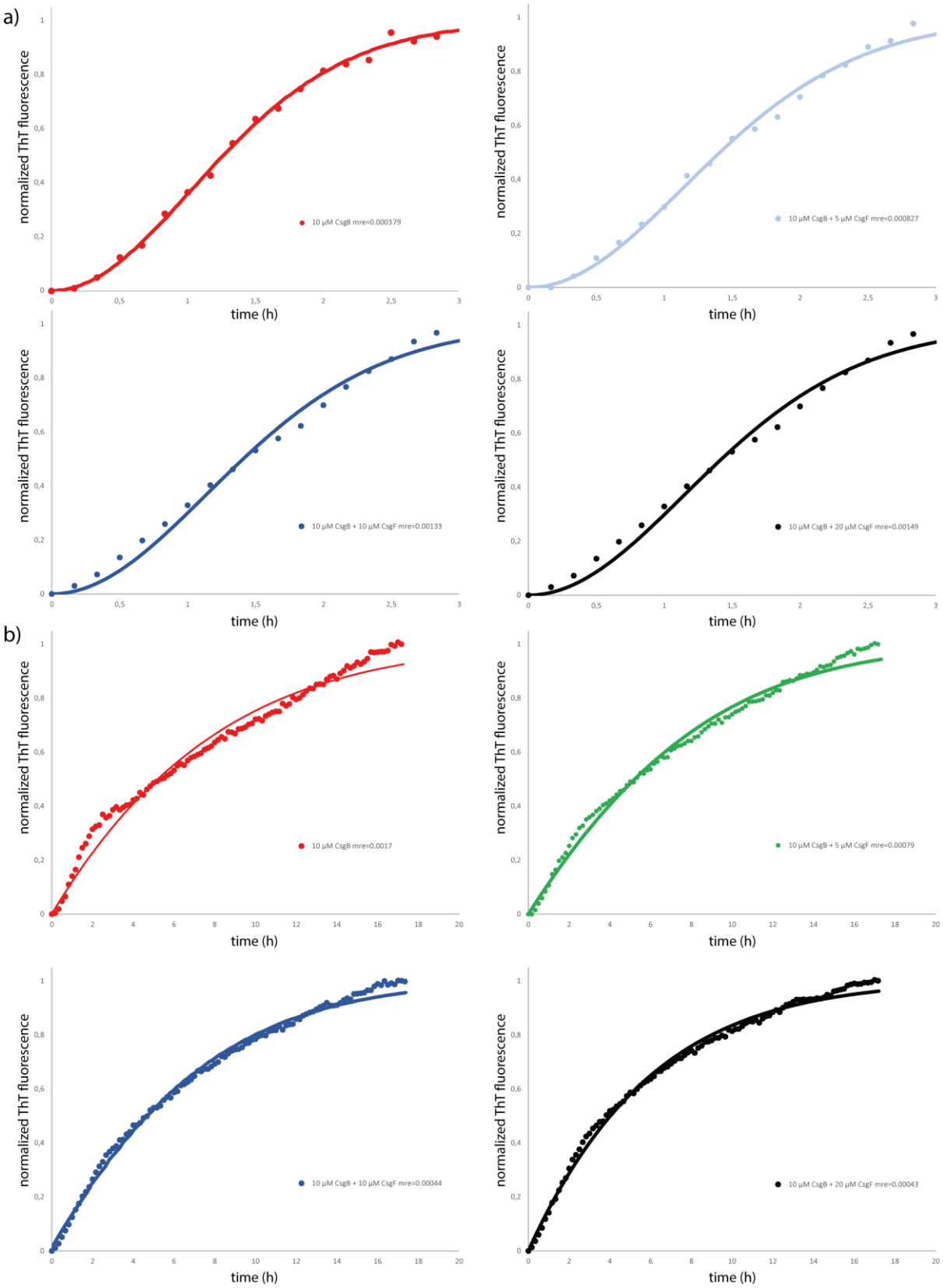


Figure S5: Fitting of the Thioflavin T aggregation profiles using the unseeded (a) and the seeded (b) nucleation-elongation model of the Webtool Amylofit, applied to the first 3 hours of aggregation (a) or the full time frame (b)

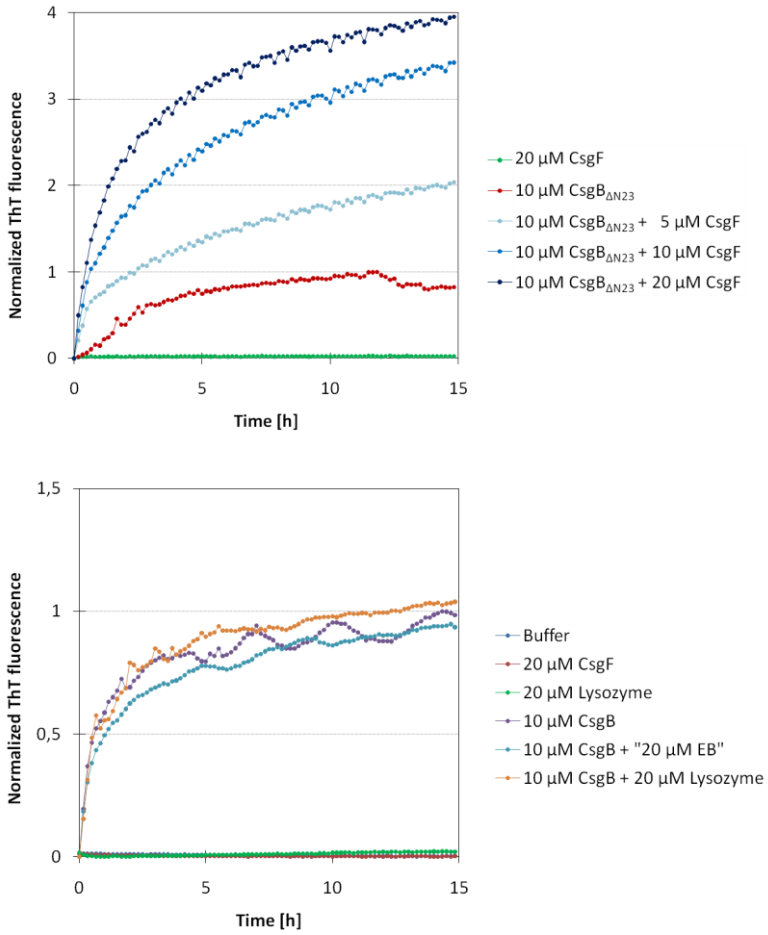


Figure S6: Additional Thioflavin T amyloid aggregation assays (EB = Elution Buffer - CHES pH 10).

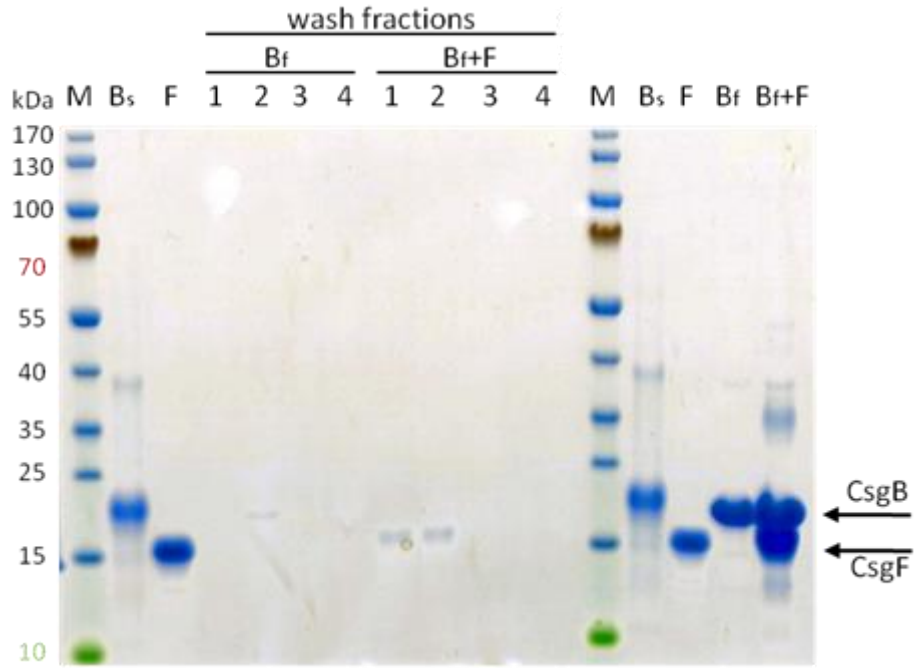


Figure S7: SDS-PAGE analysis of CsgB fibers aggregated with and without CsgF and washed with urea. M: marker; Bs: soluble CsgB serving as a control; F: CsgF; Bf: fibrillized CsgB. Washing fractions are numbered. Fibers were washed twice with 8 M urea/50 mM KPi pH 7.2 (steps 1 and 2) and two times in 125 mM Tris-HCl pH 7.5/0.8 M urea (steps 3 and 4). To dissolve CsgB fibers, the pellets were

resuspended in 6 M Guanidinium thiocyanate, 50 mM PBS pH 7.2 and precipitated with TCA (trichloroacetic acid).

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CLUSTAL W (1.83) multiple sequence alignment

sp|CSGA_20-41   GVV------PQYGGGGNHGGGGNNSG-------------------P
sp|CSGF_19-65   GTMTFQFRNPNFGGNPNNGAFLLNSAQAQNSYKDPSYNDDFGIETP
                *.:      *::**. *:*.   **.                   *
CLUSTAL W (1.83) multiple sequence alignment

sp|CSGF_19-65   GTMTFQFRNPNFGGNPNNGAFLLNSAQAQNSYKDPSYNDDFGIETP---
sp|CSGB_21-41   AA-GYDLAN---------------S-E-----------YNFAVNELSKS
                .:  ::: *               * :            :*.::
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Figure S8: Sequence Alignment of N-terminus of CsgF with CsgA or CsgB.

Supplementary References

Bax A, Clore GM, Gronenborn AM (1990) 1H□1H correlation via isotropic mixing of 13C magnetization, a new three-dimensional approach for assigning 1H and 13C spectra of 13C-enriched proteins. J Magn Reson 88:425–431. doi: 10.1016/0022-2364(90)90202-K

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Kay LE, Ikura M, Tschudin R, Bax A (1990) Three-dimensional triple-resonance NMR spectroscopy of isotopically enriched proteins. J Magn Reson 89:496–514. doi: 10.1016/0022-2364(90)90333-5

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